and C6 Retro-LfB6 **(C6-RWQMeWRR-NH_2)** were examined by solid-state ^{31}P and ^{2}H NMR in mechanically aligned bilayers composed of a neutral membrane with a zwitterionic head group (DMPC) and a bacterial-like membrane composed of a 3:1 mixture of neutral (DMPC) and anionic lipids (DMPG). ^{2}H NMR spectra suggest weaker binding or less well-defined orientations of the Retro-LfB6 peptides compared to native LfB6 peptides. ^{31}P NMR spectra reveal that the lipids remain primarily in a bilayer arrangement, with the peptides causing little change to the phosphate head groups. Antimicrobial assays demonstrate that the C6-acylated, Trp-methylated, Retro-LfB6 peptide has enhanced activity relative to the native peptide against *S. aureus*. Results from partitioning assays will be compared with the NMR and antimicrobial data.

2781-Pos Board B767

Lactoferricin Peptides Characterized Using All-Atom Molecular Dynamics Simulations and Solid State NMR

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Lactoferricin B is a cationic antimicrobial peptide with broad spectrum effectiveness. A small hexapeptide (LfB6, RRWQWR-NH2) extracted from this peptide has similar antimicrobial properties that can be enhanced by attaching a short fatty acid to the N-terminus (C6-LfB6). The mechanism for interaction between the antimicrobial peptide and the bacterial cell membrane is not well understood, but it is hypothesized to depend on lipid composition. Bacterial membranes generally contain a significant (20-25%) fraction of negatively charged lipids, in contrast with the zwitterionic mammalian membranes. In the case of LfB6, the presence of the tryptophans and arginines is thought to promote selective interactions with the negatively charged bacterial membranes. Here, we investigate the interactions of both LfB6 and C6-LfB6 with lipid bilayers using all-atom molecular dynamics simulations in concert with solid state 2H NMR. In particular, we investigated the peptide interactions with a model bacterial membrane (3:1 POPE:POPG) and a model mammalian membrane (POPC), and compared our results to solid state 2H NMR data. The results show subtle changes in the membranes and conformational substates of the lipopeptides, elucidating the effects of antimicrobial peptide binding.

2782-Pos Board B768

The Effects of Membrane Curvature on the High-Resolution Structures of Membrane-Bound Antimicrobial Piscidin 1 and Piscidin 3

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First discovered in the mast cells of hybrid striped sea bass, piscidin 1 and piscidin 3 (p1 and p3) are two isoforms of the piscidin family of antimicrobial, cationic, and amphipathic peptides (ACAPs) that have demonstrated broad spectrum activity against both Gram-positive and Gram-negative bacteria. Although recently identified as being active against HIV-1, p3 has lower antimicrobial and hemolytic activity than p1 in general. In the presence of membrane-mimetic lipid bilayers, both p1 and p3 adopt cationic, amphipathic, alpha-helical structures, which are critical for the specificity of membrane binding and subsequent cell lysis. However, the precise mechanism of action and the structural factors that lead to differences in activities of these peptides remain unclear.

Previously, the structure of p1 and p3 was elucidated in 3:1 DMPC: DMPG bilayers that mimic the charge of the E. coli cell membrane while reducing membrane curvature. Here, 1:1 POPE: POPG phospholipid bilayers were used since this composition closely mimics the negative charge and curvature of the phospholipid membranes of Bacillus cereus. Oriented bilayer samples of 1:20 peptide:lipid were analyzed using high-definition solid state NMR, which yields 15N-1H dipolar coupling as well as 15N and 1H chemical shifts for each labeled residue. This data provided structural constraints for the computational determination of a high definition atomic structure of each isoform at the water-bilayer interface.

By comparing the structural differences of p1 and p3 in various membrane curvatures to the activities of the peptides against bacteria with the same membrane composition, specific structural moieties that are critical to the antimicrobial activity can be determined. Understanding the particular interactions between the peptide with the membrane that lead to cell death is an important step for designing novel antibiotic pharmaceuticals.

2783-Pos Board B769

Membrane Penetrating Leukotoxin of *Aggregatibacter Actinomycetemcomitans* have High Affinity for β Integrin Cytosolic Tail

Patrik Nygren.

Aggregatibacter actinomycetemcomitans secrets a leukotoxin (LtxA), a member of the repeats-in-toxin (RTX) family, which kills human leukocyte function-associated antigen-1 (LFA-1; α_L/β_2)-bearing cells. The toxin interacts with the α chain extracellular region but not β. Using surface plasmon resonance (SPR) it was possible to show that the LtxA binds to the β cytosolic domain with high affinity. This LtxA-cytosolic β chain interaction might be part in the cytotoxicity of the leucotoxin. Earlier studies of LtxA have shown that it induces the formation of large pores in the cell membrane, which in turn causes leakage. A further study of this internalization of the LtxA into cells was done with flow cytometry, using Jurkat cells treated with FITC labeled LtxA. Although the FITC labeled leucotoxin is internalized it does not kill cells. This indicates that the lysine residues in the LtxA are highly important for the cytotoxicity but does not have a crucial role in the protein-membrane interaction.

2784-Pos Board B770

Characterizing a Detergent-Like Commonality Among Antimicrobial Peptides with Structural and Mechanistic Differences

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Largely distributed among living organisms, antimicrobial peptides are a class of small (<100 amino acid residues) host defense peptides that induce selective membrane lytic activity against microbial pathogens. The permeabilizing behavior of these diverse peptides has been commonly applied to the formation of pores, categorized as the barrel-stave, toroidal, or carpet type. With the continuing discovery of new peptide species, many are uncharacterized and the exact mechanism is unknown. Through the use of atomic force microscopy, we have previously shown that the disruption of mica supported lipid bilayer patches by protegrin-1, an 18-residue, cationic, beta sheet antimicrobial peptide isolated from pig leukocytes, is concentration dependent. The intercalation of antimicrobial peptide into the bilayer results in structures beyond that of pore formation including the observance of worm-like micelles. This suggests that antimicrobial peptide acts to lower the interfacial energy of the bilayer in a way similar to detergents. We pose that antimicrobial peptides act universally within a detergent-like mechanism in which pore formation is a small part of a much more complex phase diagram that spans peptide-lipid aggregates beyond that of lamellar bilayers. Antimicrobial peptides with structural and mechanistic differences have been studied and current results with magainin-1 and aurein 1.1 for example exhibit a mechanistic commonality. Future aspirations of antimicrobial peptides as new sources of antibiotics may be realized as a universal mechanism can streamline the synthesis of de novo designed peptides.

2785-Pos Board B771

Toxin Insertion Into Lipid Monolayers at the Air/Water Interface is Promoted by the Cognate Receptor Protein

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Bacterial surfaces mediate many interactions important for health. We describe the production and analysis of a membrane protein containing lipid monolayer, at the air-water interface. Monolayers were formed by spreading a solution of outer membrane protein F (OmpF) / DPPG vesicles at the air-liquid interface. Monolayer structure was analysed parallel and perpendicular to the interface using surface pressure isotherms (SP), Brewster angle microscopy (BAM), see figure, and neutron reflectometry (NR). The domain structure of an OmpF containing monolayer was distinct from that of the protein free monolayer over all surface pressures. OmpF is the receptor for the antibacterial toxin colicin N (ColN) and, by an unknown mechanism, enables the toxin to translocate across the bacterial outer membrane barrier in order to kill E. coli cells. SP and NR data indicated that OmpF enabled ColN to penetrate deeply into the anionic lipid monolayer, and BAM showed a more selective interaction than that seen for lipid monolayers. This suite of methods including selective deuteration can thus provide membrane penetration data for a range of receptor-mediated translocation events in biology where resolution in multiple axes is required.